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Similarity of salivary microbiome in parents and adult children

Kati Sundström [Corresp., 1, 2], **Pashupati P Mishra** [1, 2], **Mikko J Pyysalo** [3, 4, 5, 6], **Terho Lehtimäki** [1, 2], **Pekka J Karhunen** [1, 2], **Tanja Pessi** [6]

[1] Faculty of Medicine and Health Technology and Finnish Cardiovascular Research Center, University of Tampere, Tampere, Finland

[2] Fimlab laboratories ltd., Tampere, Finland

[3] Department of Otorhinolaryngology, Faculty of Medicine and Health Technology, University of Tampere, Tampere, Finland

[4] Department of Oral and Maxillofacial diseases, Tampere University Hospital, Tampere, Finland

[5] Oral Health Services, City of Tampere, Tampere, Finland

[6] Department of Molecule Microbiology, Faculty of Medicine and Health Technology, University of Tampere, Tampere, Finland

Corresponding Author: Kati Sundström
Email address: kati.sundstrom@uta.fi

Background: Human saliva contains approximately 700 bacterial species but the relatedness of salivary bacteria from parents to adult children is not investigated in humans. The objectives were to investigate the entirety of salivary bacterial DNA profiles and whether and how families share these profiles and also compare these communities between adult parent-off-spring pairs using 16S rRNA gene amplicon sequencing.

Results: The most abundant phyla in two separate families were *Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria* and *Actinobacteria*. Family ties explained 13 % of the variance between individuals' bacterial communities (R^2=0.13; P=0.001). Mothers shared more OTUs with their adult children compared to fathers, but this linkage seemed to be weaker in the family with older adult children. We identified 29 differentially abundant genus level OTUs (FDR < 0.05) between the families, which accounted for 31 % of the total identified genus level OTUs

Conclusions: Our results indicate that adult family members share bacterial communities and adult children were more similar to mothers than fathers. Our results suggest implicitly that a similarity in oral microbiome between parent-child pairs is present, but may change over time.

Background

The human body is considered as holobiont, meaning an organism consisting of the host and all its associated microbiota (1), which consists of approximately $3.0 \cdot 10^{13}$ human cells and $3.8 \cdot 10^{13}$ bacterial cells (2). Bacteria are transmitted to the host in two ways: horizontal transmission occurs between unrelated individuals by contact or by respiratory, oral or fecal spread. Vertical transmission occurs directly from parents to offspring, *e.g.* via ovum, placenta, vagina, milk or saliva (3,4). According to the holobiont theory, where humans are a combination of host and microbial cells, birth is more than just an origin of a new individual; it is an origin of a new community, *i.e.* a new holobiont (5).

The holobiont theory is supported by studies on meconium, the first stool of a mammalian infant, which is secreted during foetal time and shown to contain bacteria (6). Jimenez et al. (7) showed in mice studies that labelled *Enterococcus faecium* was found in the pup's meconium after an aseptic caesarean section in those pregnant mice whose diet contained the same bacteria. Infants acquire their mothers' microbiota from multiple anatomic sites after birth. From the birth canal, the child obtains the mother's vaginal and faecal bacteria (1) and bacteria from milk during breastfeeding (8). It has been shown that the diversity of a new-born's gut microbiome changes gradually over time, reflecting changes in diet (9).

The oral cavity is a major gateway for bacteria to enter the human body and a natural route for passage to respiratory and gastrointestinal tracts. The oral cavity consists of a diverse and complex community containing hundreds of different bacterial species. Saliva is a good candidate to study human microbiota since the sampling is non-invasive and fast. Salivary microbiota can also be distinguished from other oral microbiomes, such as gingival or tongue microbiome (10). It contains approximately 700 different bacterial species (11) at an average density of 1.4×10^8 organisms per millilitre (12). Due to the abundance of bacteria and its' distinguished characteristics, it is easy to build up individual bacterial profiles. Moreover, the microbiome in the mouth is considered more stable than the one in the gastrointestinal tract and other microbial sites of the body (13). A longitudinal twin study showed that there is a core oral microbiome that does not change over time, but also that there is no difference between monozygotic and dizygotic twins, indicating that genetics do not affect oral microbiome composition (14). However, the similarity of the oral bacterial microbiome among adult family members is poorly known, and whether this bacterial microbiome profile characterizes families.

Our aim was to study the relatedness of oral microbiome by amplifying the 16S rRNA gene from salivary samples and to evaluate if similarity of salivary bacterial profiles is observed in parents and their adult children and to assess the difference in bacterial community between parents and their adult children.

Materials and methods

Study population

The study subjects were a family of three generations including ten adults, and an unrelated family of two generations including four adults (Figure 1) (ethical approval by the Regional Ethics Committee of the Expert Responsibility area of Tampere University Hospital, reference number: R12217, and oral consent). Subjects were asked using a questionnaire about their general health, smoking habits and living conditions. No DNA tests have been made to confirm relatedness, but there are no reasons to doubt it. All adult children have shared household with their parents at least until the age of 18 years. Both families live in the same area in Southern Finland in an urban or suburban setting. All subject's living style, eating habits and healthcare have stayed similar to their family, they also still frequently visit their family. All sampled subjects were used to study the entirety and total bacterial genera of oral microbiota using NGS.

Figure 1. A pedigree of the population used in this study. Family 1 (subjects 1-10) is located on the left and family 2 (subjects 11-14) on the right. The squares denote males and circles females, sample numbers are marked inside and ages (y) below the circles/squares.

Collection of saliva samples

Unstimulated saliva samples were collected into sterile plastic vials (Sarstedt AG & Co, Nümbrecht, Germany). Samples were stored at -20°C and analysed within 18 hours. The subjects were asked to not eat, drink or smoke (subject 13 was the only smoker) for two hours prior to sampling.

DNA extraction and sequencing of the 16S rRNA gene

DNA was extracted from a maximum volume of 2 ml saliva according to the PureLink microbiome DNA purification kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). All samples were amplified in triplicates using universal primers targeting the V3-V4 regions on 16S rRNA gene: the forward primer with adapter was **341F** TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGA GGC AGC AG (15) and the reverse primer with adapter was **R806** GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT (16). The reaction mixture (25 µL) contained 2.5 µl genomic DNA, 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA), and 0.2 mM forward and 0.5 mM reverse primer. The PCR reaction conditions for amplification of DNA were as follows: Initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 20 sec, annealing beginning at 65°C and ending at 55°C for 15 sec, and extension at 72°C for 30 sec. The annealing temperature was lowered 1°C every cycle until reaching 55°C, which was used for the remaining cycles. Final elongation was for 5 min at 72°C. Negative controls were included in triplicates during amplification. Magnetic bead purification (Beckman Coulter, Brea, California, USA), second PCR, normalization and pooling were performed according to Illumina's 16S metagenomic

sequencing library preparation protocol (Illumina ltd., San Diego, California USA). MiSeq® Reagent Kit v3 for 600 sequencing cycles (Illumina ltd. San Diego, California) was used for MiSeq library with a final concentration of 4 pM and with 10 % PhiX control. The DNA pool included a commercial *Streptococcus mitis* strain (ATCC® 49456™, LGC Standards, Teddington, Middlesex, UK) as mock community.

Data analysis

The analyses were performed with Quantitative insight to microbial ecology (QIIME) (17) and Mothur software (18). Low quality sequences were trimmed with minimum average PHRED quality score threshold of 20 (Q20) using Trimmomatic version 0.33 (19). Sequences shorter than 200 nucleotide bases were dropped out. Chimeric sequences were identified using usearch61 (20) method in *de novo* mode via identify_chimeric_seqs.py script in QIIME 1.9.1. Contaminants, *i.e. Archaea, Eukarya*, mitochondrial and unknown sequences were filtered out with remove.lineage command in Mothur (version 1.38.1.). Taxonomies that were different among replicates were considered as bacterial contamination and were removed separately from each sample.

OTU picking was done with QIIME (version 1.9.1.) with UCLUST (20) in *de novo* mode via the pick_otu.py script. Default parameters were used and clusters were generated with 97 % similarity threshold but we focused our report to genus level based on assigned taxonomy to OTUs. Taxonomy assignment was done to the representative sequences for each of the OTUs via assign_taxonomy.py script against SILVA database (123 release) (21) as well as Human oral microbiome database (HOMD) (version 14.51) (22) using default parameter settings. Similarly, alignment of the representative sequences, filtration of the gaps present in all sequences in the alignment and building of a phylogenetic tree using the alignment were accomplished using the scripts in QIIME 1.9.1 software suite. Picked OTUs were converted to an OTU table in BIOM format for subsequent analysis using make_otu_table.py script. The OTU table was normalized using cumulative sum scaling (23) via normalize_table.py script in QIIME 1.9.1.

Beta diversity was calculated using unweighted UniFrac method via beta_diversity.py script. Adonis test (24) was performed to assess the difference in bacterial community between the two families. Homogeneity of dispersion among groups and the validity of Adonis was tested using PERMDISP method (25) via compare_categories.py script in QIIME 1.9.1 software suite. Differential abundance analysis was done using DESeq2 method via differential_abundance.py script in QIIME 1.9.1. Two nuclear families from our cohort were chosen to investigate the difference in OTUs shared between mother or father with adult children using Venn diagrams in R. Significance of the overlaps were estimated with hypergeometric test. Nuclear family A consists of parents and three daughters (subjects 1,2,4,5 and 6) and nuclear family B consists of parents, one son and one daughter (subjects 3,4,7 and 8) (Figures S3-S5).

Results

The quality control rate, Q30 %, for the run was 75.7 %. The total number of sequences obtained in one single run from the analyzed samples was 5 293 569, the average number of reads per sample was 182 536, and the average Shannon species diversity was 2.997 (SD=0.108). According to the technical data from the sequences, sample 13 was left out from the data analysis due to the low number of reads after pre-processing. Family transmission study was conducted in Family 1 for subjects 1-8 because only core families with both father, mother and all adult children present can be used for family studies. Subjects 9 and 10 from family 1 were not used for transmission study because they had two different fathers and comparison could not be made. Family 2 was not used for transmission study due to lack of father. Moreover, even if dental health was not examined in detail before sampling, no subjects apart from subject 11 claimed oral disease and no signs of oral disease could be found in bacterial DNA analysis in other subjects.

Differential abundance analysis of SILVA based taxonomy yielded 69 oral taxa. The analysis was repeated with HOMD based taxonomy, which yielded 91 taxa. According to SILVA, the major phyla were *Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria* and *Actinobacteria*. (38 % of the total identified phyla). The most common genera were *Streptococcus spp., Veillonella spp., Prevotella spp., Neisseria spp.* and *Leptotrichia spp.* (3.7 % of the total identified genera). The most significant abundances were observed in bacterial taxa like unclassified *Synergistaceae, Atopobium spp.,* Human oral bacterium BD1-5*, Lactobacillus spp.* and *Butyrivibrio spp*. (Table 1).

Differences in oral microbiome between and within families

The R^2 value obtained from Adonis test indicates that approximately 13 % of the variances in the distances is explained by grouping based on families (R^2=0.13; p=0.001). Significant difference in dispersion was indicated between the two families by PERMDISP test (F-value=9.17, p=0.006).

Of the 69 oral taxa detected by Differential abundance analysis in SILVA based taxonomy, 29 were significantly different in two families (FDR <0.05, Supplementary Material, table S1). Of the 91 taxa detected with HOMD based taxonomy, 39 were found to be significantly different (FDR <0.05, Supplementary Material, table S2). Of all observed taxa, 22 were common to both databases. Major differences were observed in unclassified taxa (n = 6, supplementary material, tables S1 and S2)

Table 1. Five most abundant taxa and their differences compared between the two families and obtained with SILVA. $P_{adjusted}$ is the adjusted p-value; Stat is the measure by how much a certain taxon is different between the families.

Operational Taxonomic Unit	$P_{adjusted}$	Stat

Synergistaceae unclassified	$1.66*10^{-11}$	– 7.381
Atopobium spp.	$6.28*10^{-9}$	6.441
Human oral bacteria BD1-5	$8.21*10^{-8}$	– 5.973
Lactobacillus spp.	$1.84*10^{-7}$	5.792
Butyrivibrio spp.	$1.07*10^{-4}$	– 4.492

Shared OTUs between parents and adult children were mapped in Venn diagrams (Supplementary Material, figure S3. Overlaps with hypergeometric P-value were analyzed, indicating adult children share more OTUs with mothers as compared to fathers but the difference in shared OTUs decreased over time with the child aging (Figure 2, Table 2).

Figure 2. Shared OTUs between parents and adult children according to Figure 1

Table 2. The parent-child-pairs OTU overlap and hypergeometric p-value.

Nuclear family A	**Age of child (y)**	**Age of parent (y)**	**Overlap (number of OTUs)**	**Hypergeometric P-value**
Mother 2-daughter 4	51	76	54	0.006811284
Mother 2-daughter 5	50	76	39	*NS*
Mother 2-daughter 6	53	76	52	4.593393e-05
Father 1-daughter 4	51	82	52	3.138095e-05
Father 1 – daughter 5	50	82	37	*NS*
Father 1-daughter 6	53	82	46	0.0001144497
Nuclear family B	**Age of child (y)**	**Age of parent (y)**	**Overlap (number of OTUs)**	**Hypergeometric P-value**
Mother 4 - daughter 7	22	51	60	2.649257e-06
Mother 4 - son 8	20	51	62	*NS*
Father 3 - daughter 7	22	54	39	0.0005486819
Father 3 - son 8	20	54	42	*NS*

Discussion

Saliva is one of the most studied oral microbiomes in humans due to the ease of collection (26). We used next generation sequencing and two databases (SILVA, HOMD) to analyze the entirety and vertical transmission of bacterial community in saliva in two families. The most significantly abundant taxa according to SILVA, *Synergistaceae* unclassified, was not recognized by HOMD. Among all significant taxa, 22 same taxa were observed in both databases, among the 10 most significantly abundant taxa *Peptostreptococcaceae, Megasphaera* spp., *Capnocytophaga* spp. and *Slackia* spp. were recognized in both databases. Overall, the taxa recognized by the databases were relatively similar, but their RFD-values were for the most part not consistent.

Of the two databases, SILVA is older, and for long considered as the gold standard. SILVA provides updated data sets of aligned small (16S/18S) and large subunit (23S/28S) sequences for all three domains of life (*Bacteria*, *Archaea*, and *Eukarya)* (27), whereas HOMD is a relatively new database, but has lately been used a lot in oral microbiome related articles (28-30). HOMD is a smaller database, since the human oral cavity only consists of approximately 700 species, whereof 400 are currently listed in HOMD. It is possible to go down to species level with this phylogeny-curated database (31). The variety between results from databases can partly be explained by the biases for each database, where certain bacteria genera or phyla is often overrepresented, but also since assigning down to genera level from 16S rRNA gene sequences can hide micro-heterogeneity and thus falsify OTU results (32). A set of universal primers was used for amplification of bacterial DNA, however, no primer pair is actually universal, and thus there is a possibility of DNA sequence dropout due to the primers, which are not amplifying all sequences.

The main reason for similarity of microbiota between newborns and mothers is considered bacteria that relocate from the birth canal during labor and from breast milk in infancy (33). The earliest colonizers in the child's oral cavity depend on both surrounding microbes and antibodies inherited from the mother. Thus, the greater similarity of maternal bacteria dates back to childhood and a close physical contact between mother and infant. In contrast, to our knowledge, the stability of bacterial transfer has not been studied in adulthood between adult mother-child pairs over generations or by comparing oral microbial profiles of the adult child to the father's microbiome. Previous studies (34-36) have focused on the development of the microbiome in children and adolescents, but not on the resemblance of the microbiome in adulthood, as we have now done.

A study based on microbiome analysis of twin-pairs concluded that environmental factors provide the greatest influence on oral microbial composition (14). Kort et al. (37) however, showed that intimate kissing increases similarity between oral microbiomes of couples only temporarily, suggesting that an adult's microbiota is stable. In our study, oral bacterial profile comparison between parents and younger adult children show a higher resemblance compared to elderly parents and their older adult children. Younger adult children, 7 (22 years) and 8 (20 years), still live with their parents, and this could partly explain the larger amount of shared OTUs. Older adult

children 4 (51 years), 5 (50 years) and 6 (53 years) have lived in their own households for at least two decades.

It has been suggested that a large part of the oral microbiome is similar around the world (38). Our PERMDISP results suggest that there are certain differences between families, and those differences might be due to the difference in dispersion instead of center. Moreover, the Adonis result reporting 13 % variance due to family ties and rest due to environmental bacteria in the mouth is consistent with studies by Kort et al. and Nasidze et al (37,38).

The major weakness of this study is, however, the sample size. The small sample size interferes especially the heterogeneity calculated using PERMDISP and due the fact that we had only a few parents-adult children pairs to draw Venn diagrams and to calculate hypergeometric p-values. Thus, we conclude that larger cohorts are needed to confirm our preliminary results.

Conclusion

In conclusion, our exploratory study suggests that even if mothers could be closer to their adult children compared to fathers in early adulthood, this similarity may change over time. Our study suggests that even though the oral cavity is very prone to inhabit environmental bacteria, the mother still has a role in her offspring's oral microbiota in the adulthood. This research setting can serve as a foundation for further research with larger sample sizes and better defined families.

List of abbreviations

NGS: Next generation sequencing OTU: Operational Taxonomic Unit FDR:

QIIME: Quantitative insights to microbial ecology Q20: Quality score threshold of 20

HOMD: Human oral microbiome database PCA: Principal component analysis

SD: Standard deviation 16S/18S: Small subunit of rRNA 23S/28S: Large subunit of rRNA NS: Non-significant

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Supporting information

Table S1. Bacterial taxa with adjusted P-values obtained from SILVA. P-values are adjusted for multiple testing correction in order to reduce false positive results

Operational Taxonomic Unit	Stat	$P_{adjusted}$
Synergistaceae unclassified	-7.3808154872676	$1.66762284764966*10^{-11}$
Atopobium spp.	6.44115283003831	$6.28417833059817*10^{-9}$
Human oral bacteria BD1-5	-5.97344706711132	$8.20765704263695*10^{-8}$
Lactobacillus spp.	5.79192208476946	$1.84401376196454*10^{-7}$
Butyrivibrio spp.	4.49192635861786	0.00010744826924249
Peptostreptococcaceae Unclassified	-4.4141529062156	0.000134363306731825
Solobacterium spp.	4.37725914643883	0.000141546570828339
Megasphaera spp.	3.82639216611577	0.00125306525070144
Capnocytophaga spp.	-3.71622930326129	0.00178625965271426
Slackia spp.	3.68935550987769	0.00183317110352649
Bifidobacterium spp.	3.60381542833573	0.00207746602402593
Prevotella spp.	3.62774342664091	0.00207746602402593
Moryella spp.	3.61479440791409	0.00207746602402593

Clostridiales uncultured family	-3.47069906446576	0.00318961870678213
Eikenella spp.	-3.44468553682608	0.00318961870678213
Treponema spp.	-3.45549391277486	0.00318961870678213
Actinomyces spp.	3.40579549660307	0.00349649354877567
Candidate division SR1	-3.15114617344019	0.00820899720377545
Peptococcus spp.	-2.95271153098626	0.0151770882544323
Anaeroglobus spp.	-2.9085185050637	0.0167362793442853
Dialister spp.	2.85002300816242	0.0185356122414987
Pasteurellaceae unclassified	-2.85262356650637	0.0185356122414987
Fusobacterium spp.	-2.76470473390749	0.0223677043943404
Actinobacillus spp.	2.57588363170221	0.037851195377557
Mogibacterium spp.	2.54009698023371	0.0405072547191099
Cryptobacterium spp.	2.52546116174981	0.0408264485245026
Clostridiales Family XIII unclassified	-2.50630790703424	0.0417158992705353
Lachnospiraceae unclassified	-2.47339682099731	0.0443329908323991
Staphylococcus spp.	2.45949550217167	0.0446910265024849
Mycoplasma spp.	-2.32425433737055	0.0609102245083872
Bifidobacteriaceae unclassified	2.27507122456351	0.0656101540044277
Neisseria spp.	-2.15476249447586	0.0847467949973194
Streptococcus spp.	2.08339956023919	0.0986192685632586
Tannerella spp.	-1.9615996339307	0.128774784951411
Actinobaculum spp.	1.94095374693255	0.131744664494361
Veillonella spp.	1.9313192009483	0.131744664494361
Porphyromonas spp.	-1.89714731105984	0.139265928252837
Candidate division TM7	1.88168191558443	0.141048821322945
Clostridiales Family_XIII unclassified	1.864544117002	0.143434895958703
Corynebacterium spp.	-1.80705250546809	0.159573060732671
Prevotellaceae unclassified	-1.77289174739652	0.168378010836963
Scardovia spp.	1.66123492145293	0.204932502011363
Bulleidia spp.	1.53089915624901	0.256426878609273
Aestuariimicrobium spp.	1.39437716944387	0.320362894383997
Rothia spp.	-1.35187799888229	0.32806878526881
Lachnospiraceae unclassified	-1.36132819450511	0.32806878526881
Haemophilus spp.	-1.35390259424212	0.32806878526881
Oribacterium spp.	1.31508293429885	0.342701473962645
Cardiobacterium spp.	-1.30836678277671	0.342701473962645
Neisseriaceae unclassified	1.17534636604343	0.410076680620721
Kingella spp.	-1.07336775221857	0.476337327294068
Unassigned bacteria	0.949694322694806	0.566880704788816
Gemella spp.	0.910467780594551	0.585308526389668
Veillonellaceae unclassified	0.90694216067989	0.585308526389668

Clostridiales unclassified family	-0.780380999486458	0.625675090149152
Parvimonas spp.	0.757755721252263	0.625675090149152
Catonella spp.	-0.779836525422117	0.625675090149152
Leptotrichia spp.	-0.777968461102738	0.625675090149152
Neisseriaceae unclassified	-0.771147774659251	0.625675090149152
Peptostreptococcaceae unclassified	0.723772485540723	0.645919154884058
Filifactor spp.	-0.673465518484677	0.67175980909181
Abiotrophia spp.	-0.518220350498138	0.781174158371704
Anaerococcus spp.	0.492817294386512	0.785083560778642
Streptococcaceae unclassified	0.477938965895177	0.789006194443737
Aggregatibacter spp.	-0.437353148945581	0.806398357894685
Granulicatella spp.	-0.37081639665193	0.856159937732391
Unassigned bacteria	-0.247627507194918	0.90711487588156
Unassigned bacilli	0.276687284735486	0.90711487588156
Peptostreptococcaceae unclassified	-0.288446460828179	0.90711487588156
Alysiella spp.	0.247916136351445	0.90711487588156
Firmicutes Unclassified	-0.201049173084341	0.918867342398189
Mitsuokella spp.	0.152842191111398	0.940640493335124
Shuttleworthia spp.	0.0894581802750933	0.969073097952939
Johnsonella spp.	-0.027354889049866	0.987332121183642
Flavobacteriaceae unclassified	0.215353503817065	NA
Erysipelotrichaceae unclassified	-0.14722439979293	NA
Moraxella spp.	0.0668955580300727	NA

308

309

310 Table S2. Bacterial taxa with adjusted P-values obtained from Human Oral Microbiome Database (HOMD). P-
311 values are adjusted for multiple testing correction in order to reduce false positive results

OTU	Stat	$P_{adjusted}$
Lactobacillus spp.	-6.45409479474323	$1.00158240008095*10^{-8}$
Fretibacterium spp.	6.34234307252394	$1.04096470860236*10^{-8}$
Atopobium spp.	-6.13208265092909	$2.65990457824073*10^{-8}$
GN02 [G-2]	5.82008532257433	$1.35280467284957*10^{-7}$
Solobacterium spp.	-4.88862369655286	$1.86839829221903*10^{-5}$
Butyrivibrio spp.	-4.84319234933199	$1.95913217979891*10^{-5}$
Lachnospiraceae [G-3]	4.5371194342896	$7.49508403213552*10^{-5}$
Stomatobaculum spp.	-4.33679526344865	0.000166261450318532
Peptostreptococcaceae [XI][G-7]	4.19637141529889	0.000277252560309419
Peptostreptococcaceae [XI][G-5]	4.14105930587059	0.000318049109768394
Bifidobacterium spp.	-3.85307502705096	0.000975564899313541
Megasphaera spp.	-3.79912648977577	0.00111325345556533
Capnocytophaga spp.	3.73275557978932	0.00134034347744363
Bacteroidetes [G-5]	3.6243361119194	0.00184041245953167
Bacteroidales [G-2]	3.59848871852137	0.00184041245953167
TM7 [F-1] Unclassified	-3.60108780487205	0.00184041245953167
Dialister spp.	-3.54469236163852	0.00212721084096414
Eikenella spp.	3.49462849983156	0.00242635702207807
Ruminococcaceae [G-2]	-3.27584159317344	0.00510104316088404
Bacteroidetes [G-3]	3.21313404741977	0.00601220150989153
Peptostreptococcaceae [XI][G-4]	3.20040409084809	0.00601220150989153
Prevotella spp.	-3.17401139386961	0.0062872711652855
GN02 [G-1]	3.14548739349753	0.00663241363058447
Cryptobacterium spp.	-3.10479004889174	0.0072992083770579
Treponema spp.	3.02821798378423	0.00905282091527167
SR1 [G-1]	2.97871560919919	0.0102424059954776
Actinomyces spp.	-2.91716823985772	0.0116059705730353
Alloprevotella spp.	-2.92482437708152	0.0116059705730353
Clostridiales [F-1][G-1]	2.82073852171429	0.0152000621001537
Alloscardovia spp.	-2.80686618773451	0.0152336506136489
Fusobacterium spp.	2.79856158850104	0.0152336506136489
Bulleidia spp.	-2.64303320256354	0.0236230060000929
Ruminococcaceae [G-1]	-2.62732411651554	0.0239922949032303
Slackia spp.	-2.59446988299858	0.0256346941567233
Lachnospiraceae [G-2]	-2.49811070989331	0.0328196051814825
Mogibacterium spp.	-2.41574111523442	0.0401304557074349
Staphylococcus spp.	-2.38418820617003	0.0414407847808214

Peptococcus spp.	2.39299118611006	0.0414407847808214
Peptostreptococcaceae [XI][G-2]	-2.34369763989509	0.0450414145300286
Streptococcus spp.	-2.28830932984125	0.0508748812162072
Lachnospiraceae [G-8]	2.27788129109764	0.0510120954772045
Streptococcaceae Unclassified	-2.20793794143074	0.0596874084560669
Bergeyella spp.	2.09296387089537	0.0777771847710425
Actinobaculum spp.	-2.08248285459677	0.0779875372247281
Veillonella spp.	-2.02263820812174	0.088136944358989
Peptostreptococcaceae [XI][G-1]	-1.96586295913614	0.0986288855804254
Scardovia spp.	-1.85351350821255	0.12490231588979
Porphyromonas spp.	1.81290675163069	0.133871892393926
Stenotrophomonas spp.	-1.76410047668451	0.145096489789445
TM7 [G-6]	-1.75735811457737	0.145096489789445
Neisseria spp.	1.69341416736933	0.163032406381274
Selenomonas spp.	-1.63912847084439	0.179022281156862
Corynebacterium spp.	1.62267807008069	0.181670910158161
Mitsuokella spp.	-1.57600337565595	0.195968561557164
TM7 [G-5]	1.55948304918045	0.198857319998214
Propionibacterium spp.	-1.52013502705406	0.209299818081401
Tannerella spp.	1.51538518378556	0.209299818081401
Oribacterium spp.	-1.40606026531796	0.253327123548147
Mycoplasma spp.	1.3571676687232	0.270369862914956
Veillonellaceae [G-1]	1.35214738465248	0.270369862914956
Rothia spp.	1.29260609869692	0.295828758838883
Gemella spp.	-1.14958991035011	0.371431920172595
Campylobacter spp.	-1.09521053422064	0.399286442454863
Cardiobacterium spp.	1.05179741312851	0.421032977609626
Lautropia spp.	-1.03309598970945	0.426821940776593
Pseudomonas spp.	-0.955302493025047	0.473137526312782
Olsenella spp.	-0.932631152786705	0.481984488788975
Parvimonas spp.	-0.920687501524083	0.48328899282398
Peptostreptococcus spp.	-0.797906460225923	0.566566365568136
Shuttleworthia spp.	-0.71741769712723	0.621810129840504
Mobiluncus spp.	-0.636544812741282	0.637362792923263
Bacteroides spp.	-0.674503535068853	0.637362792923263
Abiotrophia spp.	-0.622755893966515	0.637362792923263
Catonella spp.	0.682927255705345	0.637362792923263
Johnsonella spp.	0.649680787555039	0.637362792923263
Lachnospiraceae [G-7]	-0.63897851562877	0.637362792923263
Haemophilus spp.	0.631908936486933	0.637362792923263
TM7 [G-3]	-0.549758914282798	0.687033285753241
Lachnospiraceae [XIV] Unclassified	-0.504781439100348	0.714702994183027
Unassigned Other	0.429635408659645	0.754192337880151

Enterococcus spp.	0.423110132730617	0.754192337880151
Filifactor spp.	0.425683799778011	0.754192337880151
Kingella spp.	0.386162884757101	0.7752119956042
Peptostreptococcaceae [XI][G-9]	0.339503149211114	0.794696784803174
Moraxella spp.	-0.34208891440522	0.794696784803174
Ottowia spp.	0.272130020737771	0.835290169380453
Aggregatibacter spp.	-0.26644830861666	0.835290169380453
Lachnoanaerobaculum spp.	-0.244262125116703	0.843710897418313
Peptostreptococcaceae [XI][G-6]	0.20189743513477	0.868311409745663
Granulicatella spp.	0.107298439102738	0.930594099230161
Leptotrichia spp.	0.0998304338996341	0.930594099230161
TM7 [G-1]	-0.043120118205251	0.965605782204658

312

313 Figure S3-S5. Venn diagrams showing OTU overlaps between family members

Figure 1(on next page)

A pedigree of the population used in this study.

Family 1 (subjects 1-10) is located on the left and family 2 (subjects 11-14) on the right. The squares denote males and circles females, sample numbers are marked inside and ages (y) below the circles/squares.

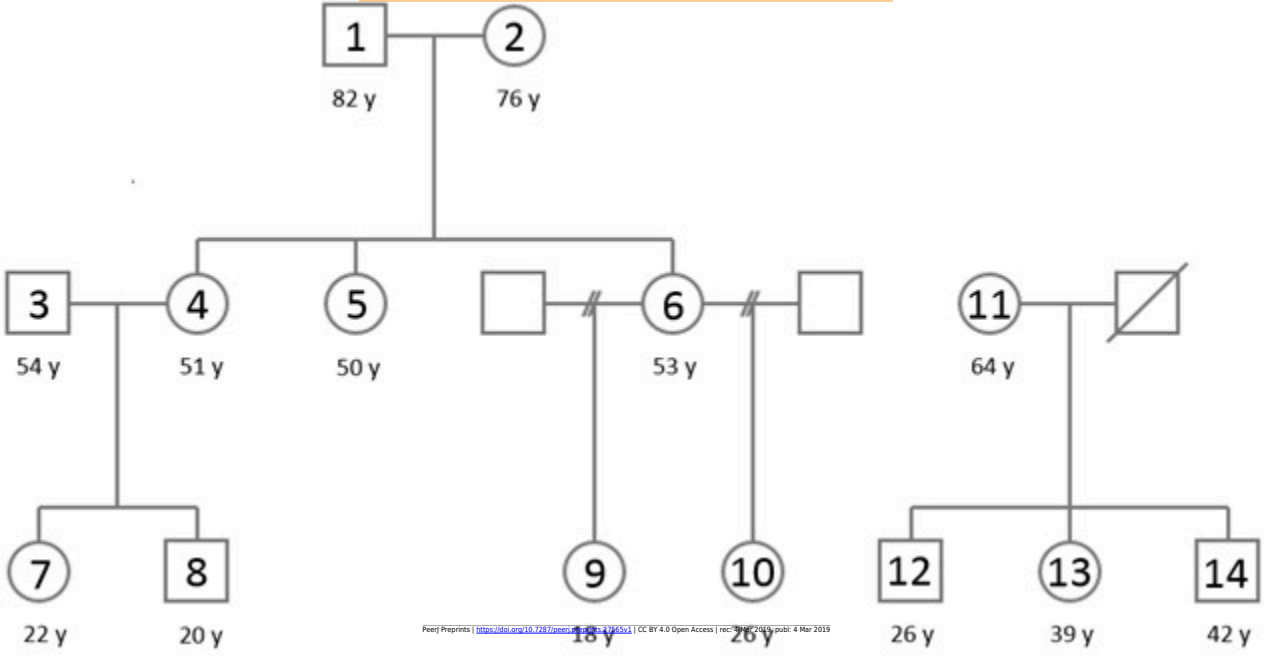

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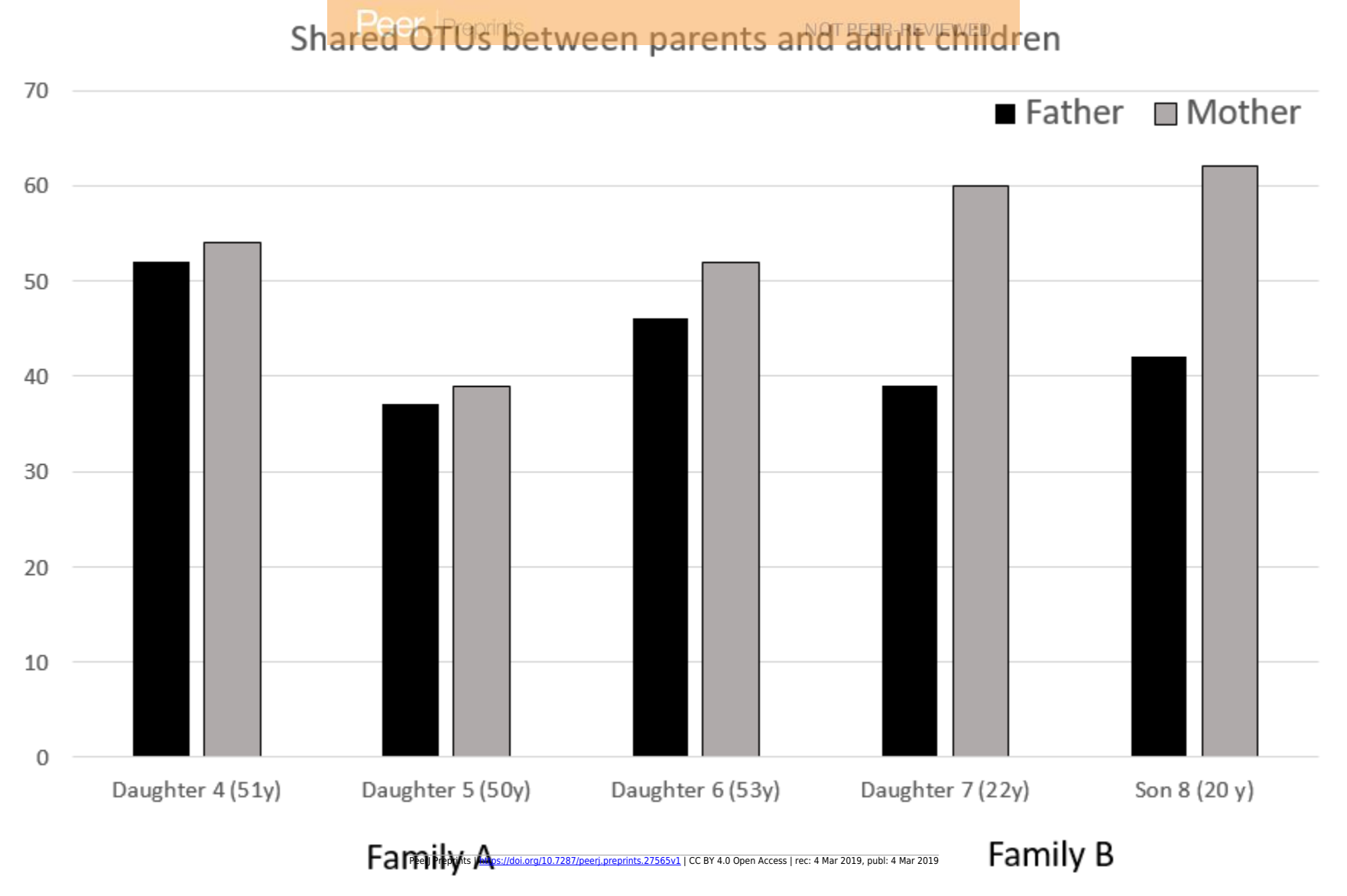
Table 1(on next page)

Shared OTUs between parents and adult children according to Figure 1



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